

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of: Gladwin et al.

Application No. 10/563,683

Filed: October 4, 2006

Confirmation No. 3225

For: USE OF NITRITE SALTS FOR THE
TREATMENT OF CARDIOVASCULAR
CONDITIONS

SUBMITTED VIA EFS

Examiner: Anna Pagonakis

Art Unit: 1614

Attorney Reference No. 4239-67618-07

COMMISSIONER FOR PATENTS
SUBMITTED VIA ELECTRONIC FILING SYSTEM

DECLARATION OF DR. BRUCE FREEMAN UNDER 37 C.F.R. § 1.132

I, Bruce Freeman, Ph.D., declare as follows:

1. I have no financial interest in the above referenced patent application and I am not a listed inventor of the invention disclosed in the above referenced patent application.

2. A copy of my *curriculum vitae* is attached hereto as **Exhibit A**. At present, I hold a position as the Irwin Fridovich Professor and Chairman in the Department of Pharmacology & Chemical Biology at the University of Pittsburgh in Pittsburgh, Pennsylvania. I have had more than 20 years of experience in research including work on the physiological effects of nitric oxide, superoxide, peroxynitrite, heme peroxidases and electrophilic lipids, and particularly the effects of these molecules on cellular function and vascular tone. I have published over 200 scientific articles in scientific journals and books, am one of the most highly quoted scientists in the world and am considered a leader in the fields of free radical biology, inflammatory reactions and the pharmacology and chemical biology of nitric oxide. By virtue of my education, training, and professional experience, I am knowledgeable about nitric oxide donors, the physiology and biology of vasodilatation, and the effects of acidified and non-acidified nitrite, as well as nitric oxide donors, on vasodilatation.

3. Sodium nitroprusside (SNP) and 3-morpholino-sydnonimine (SIN) are nitric oxide donors. Inorganic nitrites, such as sodium nitrite, are inorganic salts. Nitric oxide donors and inorganic nitrite are structurally dissimilar and they form NO in distinctly different manners. SNP and SIN release NO directly, whereas sodium nitrite interacts in chemical reactions with heme groups of enzymes and proteins to become metabolized to NO *in vivo*. I do not view that inorganic nitrites are equivalent substitutes for recognized nitric oxide donors, such as SNP or SIN, under physiological conditions *in vitro* or *in vivo*. I believe that my understanding accurately reflects the conventional wisdom in the field, especially prior to October 14, 2003.

4. I have read Zhang *et al. J. Cereb. Blood Flow Metab.*, 14:217-26, 1994 (attached hereto as **Exhibit B**) and familiarized myself with the teachings therein. This reference teaches that SNP and SIN increase blood flow and reduce brain damage in focal ischemia in experiments with rats. Prior to October 14, 2003, I would not have understood that inorganic nitrites could substitute for these nitric oxide donors to increase blood flow and reduce brain damage in focal ischemia. This belief is based on my understanding of the differences between inorganic nitrites and recognized nitric oxide donors (such as SNP and SIN).

5. As an expert in the nitric oxide field, I attest to the fact that the overwhelming evidence prior to October 14, 2003, was that non-acidified sodium nitrite was inert and not a vasodilator *in vivo*, particularly in the human circulation, and it was accepted in the pharmacology, chemistry and NO therapeutics fields of research that inorganic nitrite was an inert oxidation product of nitric oxide metabolism. This is exemplified by Lauer *et al., Proc. Natl. Acad. Sci. USA*, 98:12814-12819, 2001 (attached hereto as **Exhibit C**), a reference I have read and am familiar with, which teaches that no vasodilation occurs at venous plasma nitrite concentrations of 130 μ M (see page 12816, column 2, last paragraph) and that physiological levels of nitrite are vasodilator-inactive (see Abstract).

6. All statements made herein and of my own knowledge are true and all statements made on information are believed to be true; and further, these statements were made with the knowledge that willful false statements and like are punishable by fine or imprisonment, or both,

under Section 1001 of Title 18 of the United States Code, and that any such willful false statements made may jeopardize the validity of the application or any patent issuing thereon.

Date August 27, 2009

A handwritten signature in black ink that reads "Bruce Freeman". The signature is written in a cursive, slightly slanted style.

Bruce Freeman, Ph.D.